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Mouse brain stereotaxic injections

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An abbreviated version of this protocol was published in Science Translational Medicine in Mar 2021

Antisense oligonucleotide therapy in a humanized mouse model of MECP2 duplication syndrome

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Detailed protocol

Mouse brain stereotaxic injections - detailed protocol

1. Autoclave in advance surgery tools.
2. Inject Buprenorphine slow release 1 mg kg^{-1} (subcutaneous) one hour before the surgery.
3. Inject Meloxicam 5 mg kg^{-1} (subcutaneous) half an hour before the surgery, and for three consecutive post-operative days.
4. Turn on beads-sterilizer and heating pad.
5. Make sure to have extra clean cages for post-operative recovery. Keep the cages on the heating pad.
6. Disinfect the table area and stereotactic apparatus with ethanol 70%. Prepare the working area with all the tools necessary for surgery.
7. Check the settings of the injection pump. Set volume and injection speed.
8. Weigh the mouse and inject (intraperitoneal) with a mixture of ketamine (37.6 mg ml^{-1}), xylazine (1.92 mg ml^{-1}), and acepromazine (0.38 mg ml^{-1}) at 0.75-1.5 ml kg^{-1}.
9. Check pedal reflex (toe pinch), make sure the mouse is deeply anesthetized.
10. Once the mouse is deeply anesthetized, shave the head area.
11. Place the mouse on the stage of the stereotactic apparatus and fix the head.
12. Apply topical eye ointment.
13. Disinfect the head area alternately with betadine and 70% ethanol 3 times.
14. Using sterile gloves, make a midline incision over the skull using a sterile blade, and expose the skull.
15. Fill Hamilton syringe with the drug and place it in the syringe holder.
16. Go to Bregma with the needle and zero it.
17. Go to Lambda and check the dorsal-ventral coordinates. Change head angle until Bregma and Lambda are the same.
18. Go to the target. For intracerebroventricular injections we used the following coordinates relative to Bregma: anteroposterior = 0.3 mm, medial-lateral = 1 mm, and dorsal-ventral = -3 mm.
19. Drill a small hole through the skull.
20. Go back to bregma with the needle and zero again. Go to the target and if the drilling was in the correct place, enter the brain with the needle until the desired dorsal-ventral position.
21. Inject and wait in place for at least 5 min after the end of the injection for the drug to diffuse.
22. Gently withdraw the syringe.
23. Suture the incision and apply 3M Vetbond adhesive.
24. Inject 1ml sterile saline subcutaneous to re-hydrate the mice.
25. Take the mouse to a clean cage on the heating pad and monitor until mouse full recovers. Monitor the animal for at least 3 days.
26. Between animals, sterilize the surgery tools for 20 sec using the beads sterilizer.

How to cite:（Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used）

1. Zoghbi, H. Y., Shao, Y. and Sztainberg, Y. (2021). Mouse brain stereotaxic injections. Bio-protocol Preprint. bio-protocol.org/prep1038.
2. Shao, Y., Sztainberg, Y., Wang, Q., Bajikar, S. S., Trostle, A. J., Wan, Y., Jafar-nejad, P., Rigo, F., Liu, Z., Tang, J. and Zoghbi, H. Y.(2021). Antisense oligonucleotide therapy in a humanized mouse model of MECP2 duplication syndrome . Science Translational Medicine 13(583). DOI: 10.1126/scitranslmed.aaz7785

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